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14. ABSTRACT: This report describes progress in Year 3 of our 3-year award, which is designed to use animal models to understand how nicotine (ingested by Warfighters via smoking or chewing tobacco) affects vulnerability to develop post-traumatic stress disorder (PTSD). As reported previously, we have completed studies in which rats voluntarily self-administer nicotine to the point of dependence, receive fear conditioning (training), and are tested for fear responses 10 days later with no additional access to nicotine. This experimental design is intended to model Warfighters who use nicotine during service but later quit. We find that rats which voluntarily self-administer nicotine and are exposed to a stressor (footshock) soon after intake have abnormally reduced responses to environments previously associated with the stressor, which we term "context-potentiated startle (CPS)", but no differences in the ability to learn the association between a discrete cue (a light) and the stressor, which we term "fear-potentiated startle (FPS)". Projected to Warfighters, this suggests that self-administered nicotine is producing some anti-anxiety (beneficial) effects under these specific conditions. We also find that rats which voluntarily self-administer nicotine and are exposed to a stressor after a missed dose (i.e., during withdrawal) have abnormally persistent CPS, but no differences in FPS. Projected to Warfighters, this suggests that nicotine withdrawal is unambiguously detrimental. We now report that we have examined other permutations of our experimental design, including those in which access to nicotine is sustained for long periods of time between training and testing. Our findings with this design were conceptually similar to those when nicotine self-administration ceased: nicotine may have some beneficial effects, but nicotine withdrawal is unambiguously detrimental. Our funding period ended on August 31, 2015 before all of our experiments had been completed. We have requested a no-cost extension and have pledged to use our own discretionary resources (at no cost to the Army) to continue work on this project through December 31, 2015. On June 15, 2015 we submitted application (BA150236) that requests 2 years of support to enable us to examine if the nicotine patch, as modeled in rodents, would also have potential beneficial effects. Our findings were presented at the Substance Abuse IPR at Fort Detrick on September 30, 2015.

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INTRODUCTION

Tobacco use (smoking, chewing) is prevalent in Warfighters. Nicotine has two major effects that could influence Warfighter behavior and fitness: anti-anxiety (anxiolytic) effects that can have calming actions, and increases in alertness and cognitive function that can enhance aversive or traumatic memories. It is currently unknown if nicotine use increases or decreases vulnerability to the development stress-related illnesses such as post-traumatic stress disorder (PTSD). It is known, however, that people with PTSD are more likely to smoke when experiencing symptoms. These people report that smoking relieves their symptoms even though objective metrics indicate that it produces increases in hallmark signs of PTSD, such as elevated responsiveness to a startle stimulus (white noise bursts). It should be emphasized that nicotine effects on the development of PTSD is a separate question from whether or not people with PTSD smoke, and an important one because it represents an issue for which a research-driven policy change could affect Warfighter health.

Animal models can offer insight on whether nicotine intake affects behavioral indicators of stress. Use of animal models enables standardization of numerous important factors, including genetics, past experiences, and levels of drug (including nicotine) intake. Perhaps most importantly, animal studies can be designed to be sensitive to beneficial or deleterious effects of nicotine. This is important because if nicotine is found to have beneficial effects, there may be safer ways to administer it to Warfighters (e.g., a transdermal patch).

Our research involves a model of nicotine use (voluntary intravenous self-administration of nicotine in rats) and PTSD (fear conditioning, as reflected by fear-potentiated startle [FPS] in rats). We use FPS in rats because the same technique can be used to study PTSD in humans. It is important to emphasize that FPS in rodents is not a complete model of PTSD in humans, but it is often used to study the disorder and it does recapitulate numerous key domains—including an index trauma, persistent fearful memories, and persistent hyperarousal. Our studies have several innovative elements. In addition to the fact that that our research fills a major gap in our understanding of how nicotine might affect the development of PTSD and related behaviors, our ability to use voluntary nicotine intake in rats enables insights not possible with experimenter-delivered nicotine. In general, experimenter-administered nicotine—which can be delivered by systemic injection, by placing an animal in a passive smoke box, or by adding it to the drinking water—produces different (often aversive) responses. Most importantly, however, there is good evidence that drugs produce fundamentally different physiological effects when taken voluntarily as opposed to when it is given by the experimenter. (It is important to emphasize that it might someday be important to examine the effects of non-contingent nicotine, in case it could have therapeutic effects, but that beyond the scope of the present research.) In addition, we are able to show that the amount of nicotine voluntarily taken by our animals produces physiological dependence, as defined by the emergence of withdrawal symptoms during periods of drug abstinence. Overall, this research is intended to facilitate efforts to devise approaches that decrease new cases of stress-related illnesses in Warfighters by determining how patterns of nicotine exposure affect resilience.

This research was designed to be particularly relevant to Warfighters and thus it has numerous implications for the military. If we discover nicotine has detrimental effects, it may provide a compelling scientific justification to regulate nicotine use in the military. In contrast, should we discover that nicotine has beneficial effects, it may be possible to devise safer ways of delivering nicotine or develop new drugs that possess only the helpful effects of the drug. The outcome of our research may also be relevant to understanding how nicotine use in civilian populations affects vulnerability to developing PTSD, particularly among individuals who may routinely be exposed to stress (e.g., law enforcement, first responders).

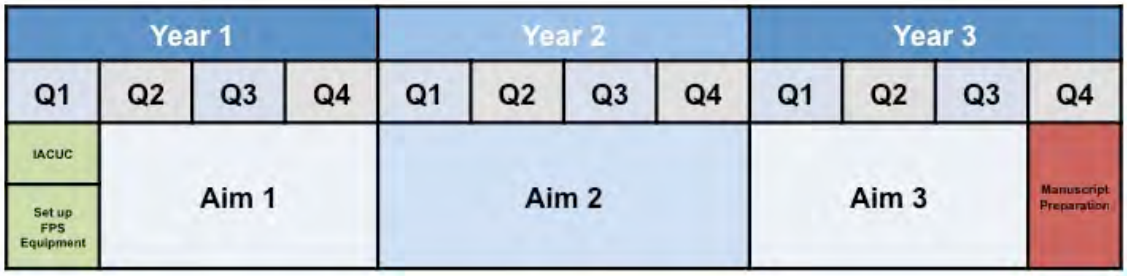
BODY

Our work provides insight on 2 basic questions of great relevance to the military. The first question (modeled in Aim 1) is whether nicotine affects the development of conditioned fear under circumstances where nicotine self-administration is discontinued after exposure to the fear-inducing stressor. These studies were intended to model Warfighters who are using nicotine during the time of the trauma but then remain abstinent leading up to a time where they encounter a stressor that triggers a stress-related memory, and data collection is complete.

The second question (Aim 2) is whether nicotine affects the development of conditioned fear under conditions where nicotine self-administration is continued after exposure to the fear-inducing stressor. These studies were intended to model Warfighters who are using nicotine during the time of the trauma and have continued to use nicotine when encountering a stressor that triggers a PTSD-related memory. Data collection is near completion; while our funding period ended on August 31, 2015, we have requested a no-cost extension. We

have also pledged to use our discretionary resources to continue this work, at no additional cost to the Army, through December 31, 2015.

We are running several months behind on these studies (see Timeline, extracted from the proposal). Specifically, we estimate that we are where we thought we would be during Year 3, Quarter 2. We have explained the reasons for the delay—such as transitions in personnel, equipment failures, damage and repairs to our infrastructure associated with the unprecedented winter 2015 season, and slow acquisition of nicotine self-administration behavior in the rats—in our quarterly progress reports. We have addressed all of these issues to the best of our abilities, but with respect to the slow pace of the behavioral studies, we feel that there are no solutions other than acknowledging that the experiments take longer than we had hoped. We feel that the outcomes have great relevance for Warfighter fitness so we do not want to implement radical changes that may have unintended consequences. Currently we do not anticipate any additional major challenges.



KEY RESEARCH ACCOMPLISHMENTS

We have accomplished all of the Year 1 goals that we described in our proposal, and have collected a data set that we feel will be of great interest to the research community. We continue to collect data for Aim 2. We have presented our findings at the 2013, 2014, and 2015 Society for Neuroscience (SfN) conferences. In addition, the PI (Dr. Carlezon) has presented these findings in-person to the Army at the Substance Abuse IPR meetings in September 2013, 2014, and 2015.

REPORTABLE OUTCOMES

Nicotine can facilitate learning and cognitive performance while also relieving feelings of stress. These actions may have opposing effects on vulnerability to stress-related illness such as PTSD. The studies supported by W81XWH-12-1-0454 were designed to examine the effect of voluntary nicotine IVSA on the development and expression of PTSD-like signs in rats using the FPS procedure. Adult male Long-Evans rats were allowed to self-administer nicotine (0.03 mg/kg/inj) in 12-hr (overnight) extended access sessions in 2-level standard operant conditioning chambers for a minimum of 14 sessions. Criteria for inclusion included IVSA of >0.7 mg/session for 4 out of 5 consecutive sessions and observable signs of spontaneous withdrawal 11.5 hrs after nicotine intake. Each rat in the nicotine exposure condition had a control counterpart that self-administered saline for an equivalent number of sessions. Rats required 21.4 ± 1.9 days (Mean ± SEM) to meet criteria for stable nicotine IVSA and received a total of 0.99 ± 0.06 mg/kg/session, which corresponds well with the range of nicotine doses known to have rewarding effects in rats; during the session that immediately preceded fear conditioning, nicotine-reinforced responding (reflected by lever-presses at the active lever) was reliably higher than saline-reinforced responding when examined at individual time points over the 12-hr time course of the last session or as an overall mean. Rats were then fear conditioned at one of two time points: either (1) immediately after or (2) 11.5 hrs after their last SA session, a time at which they were experiencing spontaneous nicotine withdrawal. Rats that had self-administered nicotine showed dependence, as indicated by increases in well-characterized spontaneous withdrawal behaviors such as ptosis and shakes/tremors. Fear conditioning consisted of 10 pairings of a 4-sec light (conditioned stimulus; CS) co-terminating with a 0.5-sec 0.6 mA footshock. Two different patterns of post-training nicotine intake were examined: for some rats, nicotine exposure was discontinued between fear conditioning and testing, whereas for others nicotine SA continued. The first pattern is intended to model military personnel who are using tobacco at the time of a traumatic event but then discontinue use (e.g., upon returning home/stateside), whereas the second pattern is intended to model military personnel who are using tobacco at the time of a traumatic event and then continue to use it. At the beginning of the fear conditioning session, match data were collected to guide group assignment such that all groups have equivalent (matched) pre-training startle responsiveness. These sessions also enabled us to confirm that neither nicotine nor nicotine withdrawal caused changes in reactivity (sensitivity) to the footshock during fear conditioning that could explain subsequent behavioral differences seen during the test sessions. At 10 days after fear conditioning, rats were tested three times, at 48-hr intervals;

during these intervals, nicotine availability was the same as used in the intervening 10 days between training and testing. We examined two metrics: %CPS (Context-potentiated startle, which reflects responsivity to the general context previously associated with the trauma); and %FPS (fear-potentiated startle, which reflects responsivity to the combination of the context plus the very specific cue that predicted the trauma).

For rats that received fear conditioning immediately after IVSA sessions plus no further nicotine exposure, nicotine has no effect on startle responsivity during match sessions. However, upon re-testing 10 days later with no further nicotine access, rats that had received fear conditioning training immediately after nicotine IVSA showed reduced %CPS and normal %FPS. This 10-day period was designed to allow nicotine dependence to resolve, as described in the literature; indeed, no signs of nicotine withdrawal were observed at the time of Tests 1-3. We interpret these data to indicate that rats that had been self-administering nicotine shortly before a traumatic event later show much less evidence of hyper-vigilance (exaggerated startle responses) than controls when placed back into the general context where they had received the trauma (i.e., CPS data). This effect is independent of the ability to learn about the trauma, which other tests show is intact (i.e., FPS data). Hyper-vigilance and elevated startle responses are disruptive to normal behavior and diagnostic criteria for PTSD in humans, and should not be confused with a healthy effect, heightened cognitive function, or enhanced readiness. Extrapolating these results to Warfighters, our findings suggest that nicotine might reduce pathological responses that occur in contexts that have broad similarities with those in which a trauma was experienced, whether in theater or after returning home. ***This would be a beneficial effect of nicotine****.*

In contrast, the pattern of results was much different for rats that received fear conditioning during nicotine withdrawal plus no further nicotine exposure. Nicotine withdrawal had no effect on startle responsivity during match sessions. However, upon re-testing 10 days later with no further nicotine access, rats that had received fear conditioning training during nicotine withdrawal showed poor extinction of %CPS and normal %FPS. We interpret these findings to indicate that when rats with reliable nicotine self-administration habits are exposed to footshock during nicotine withdrawal, they later show much more evidence of hyper-vigilance than controls when placed back into the shock-related context. Extrapolating these results to warfighters, this suggests that experiencing a trauma during nicotine withdrawal enhances the pathological responses that occur in contexts with similarities to those in which the trauma was experienced. ***This would be a detrimental effect of nicotine withdrawal (a missed dose of nicotine in nicotine users).***

A broadly similar pattern of results was seen when nicotine self-administration continued during the 10 intervening days between fear conditioning and testing. (Note that these studies are not yet finalized because they currently lack complete no-shock groups; these groups are part of the experimental design and are in progress, but as expected are indicating negligible effects in any of the tests conducted.) Again, nicotine had no effect on startle responsivity during match sessions. However, upon re-testing 10 days later after continued nicotine access, rats that had received fear conditioning training after nicotine self-administration showed brief elevation of %CPS and a brief reduction of %FPS. ***This would be a mixed effect of nicotine*****,** with improvements in some (FPS) but not other (CPS) domains.

The pattern of results was much different for rats that received fear conditioning during nicotine withdrawal. Again, nicotine withdrawal had no effect on startle responsivity during match sessions. However, upon re-testing 10 days later with continued nicotine access, rats that received fear conditioning during nicotine withdrawal showed sustained elevation of %CPS but reduced %FPS as well as enhanced extinction. When comparing the data from Aim 1 with those from Aim 2, it appears that the negative effect of experiencing a trauma during nicotine withdrawal on %CPS is retained regardless of whether nicotine access continues after fear conditioning. However, it appears that continued nicotine self-administration facilitates extinction of FPS; considering that extinction is a form of new learning that weakens the expression of old memories, this represents a potential pro-cognitive (enhanced learning) effect of continued nicotine access. ***This would be a detrimental effect of nicotine withdrawal together with a beneficial effect of continued nicotine exposure****.*

On the basis of these findings, we have applied for a new grant (BA150236, submitted on June 15, 2015) to examine whether nicotine could be used therapeutically to protect Warfighters. Smoking cigarettes and chewing tobacco are not safe methods to deliver nicotine. These “nicotine self-administration behaviors” are linked to decreases in fitness and increases in the risk of diseases such as cancer, both of which are major concerns for warfighters during and after their service. We proposed to determine if the putative beneficial effects of nicotine on contextual fear conditioning are retained when the drug is given by a different (safer)

route of administration. Nicotine patches are approved by the Food and Drug Administration (FDA) and could be easily utilized in a military context. Animal models are well-suited for answering the types of research questions described in this proposal, which are intended to provide a compelling scientific justification for studies that could be conducted in humans. Because rats are intolerant of having the patches on their skin, and their fur can interfere with drug absorption, we will approximate the nicotine patch by using subcutaneous minipumps placed underneath the skin. These minipumps can be programmed to deliver nicotine at the same rate as a patch for sustained periods of time. The pumps can also be deactivated to terminate nicotine delivery. Our studies will be designed to ask the question of whether nicotine delivered non-contingently (i.e., not by voluntary self-administration) can produce the same beneficial effects on contextual fear conditioning as when delivered contingently (i.e., by voluntary self-administration). To control these studies properly and obtain information that will be maximally useful to the military, we will examine numerous permutations, including variable degrees of nicotine exposure before and after the trauma. Our hypothesis is that we will be able to identify conditions under which nicotine can have beneficial effects when given by this safer route of administration.

CONCLUSION

To summarize our findings, the effects of nicotine depend greatly upon the precise testing conditions but in general are beneficial, including reduced anxiety-like responses when encountering contexts or cues associated with a previous trauma and reductions in anxiety-like responses that would normally be expected when a trauma is experienced during nicotine withdrawal. In contrast, the consequences of experiencing a trauma during nicotine withdrawal are unequivocally negative. The variability in our findings—the unique patterns associated with each specific procedure—likely reflect the fact that these rats all have unique individual histories of drug intake leading up to fulfilling our inclusion criteria for testing, as well as the fact that the experimental design required that some groups receive interaction with the investigator after the trauma (which might reduce anxiety-like responses) whereas others did not. *Despite these differences,* ***our findings suggest that nicotine has some beneficial effects that could be harnessed to improve warfighter health, performance, and safety, but also that nicotine withdrawal should be avoided at all costs.***

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Effects of voluntary nicotine self-administration on fear conditioning in rats

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Abstract:
Nicotine can facilitate learning and cognitive performance while also relieving feelings of stress. These two actions may have opposing effects on vulnerability to stress-related illness such as post-traumatic stress disorder (PTSD). The present experiments examined the effect of nicotine self-administration (SA) on the development and expression of PTSD-like symptoms in rats using the fear-potentiated startle (FPS) paradigm. FPS has elements that model an index trauma and enables quantification of exaggerated startle response and extinction deficits, two characteristics observed in humans with PTSD. Long-Evans rats were allowed to self-administer nicotine (0.03 mg/inj) or saline in 12hr (overnight) extended access sessions in standard operant conditioning chambers for a minimum of 14 sessions. Criteria for nicotine dependence was determined by SA of >0.7 mg/session for 4 out of 5 sessions and observable signs of spontaneous withdrawal 11.5 hrs post SA session. After criteria were met, rats were fear conditioned at one of two time points: either immediately after or 11.5 hrs after their last SA session. Fear conditioning consisted of 10 pairings of a 4-sec light (conditioned stimulus; CS) co-terminating with a 0.5-sec 0.6 mA footshock. Two different patterns of post-training nicotine intake were examined: for some rats, nicotine exposure was discontinued between fear conditioning and testing, whereas for others nicotine SA continued. At 10-12 days after fear conditioning, rats were tested immediately after SA three times, each test 48 hrs apart. Two metrics were examined in each of the test sessions: Context-potentiated startle (CPS) and Fear-potentiated startle (FPS). %CPS was expressed as the percent change in startle after exposure to the conditioning context relative to a pre-training baseline. %FPS was expressed as the percent change in startle elicited in the presence of the light CS relative to trials without the CS. Rats that received fear conditioning immediately after SA sessions plus no further nicotine exposure showed reduced %CPS and normal %FPS, whereas those that continued nicotine SA showed normal %CPS but reduced %FPS. In contrast, rats that were

fear conditioned during nicotine withdrawal plus no further nicotine showed elevated %CPS and normal %FPS. Rats that received fear conditioning during nicotine withdrawal plus continued nicotine SA also showed enhanced CPS, but reduced %FPS as well as enhanced extinction. Our data suggest that, under certain conditions, nicotine can reduce behavioral responsiveness to cues associated with a stressful (trauma-like) event, whereas nicotine withdrawal can enhance these same metrics.
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